

IN THE CLAIMS

Please amend claims 32, 34-38, 42-44, and 46. The following listing of claims replaces all prior listings.

1. (Withdrawn) A method for screening for the bioactivity of a candidate compound toward a group of related target proteins in a proteomic mixture of proteins from a cell, employing at least one probe, each probe characterized by comprising a reactive functionality group specific for said group of target proteins and a ligand and said probe, said method comprising:

combining at least one probe with an untreated portion of said mixture and with a portion inactivated with a non-covalent agent under conditions for reaction with said target proteins;

sequestering proteins conjugated with said at least one probe from each of said mixtures;

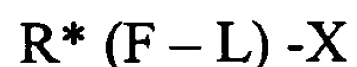
determining the proteins that are sequestered; and

comparing the amount of each of the proteins sequestered from the untreated portion and the inactivated portion as indicative of the bioactivity of said candidate compound with said target proteins.

2. (Withdrawn) A method according to Claim 1, wherein said ligand has a reciprocal receptor and said sequestering is by binding said ligand to said reciprocal receptor bound to a support.

3. (Withdrawn) A method according to Claim 1, wherein said ligand is detectable as a result of an electromagnetic signal.

4. (Withdrawn) A method according to Claim 1, wherein said probe is of the formula:



wherein:

X is a ligand for binding to a reciprocal receptor or a chemically reactive functionality for reacting with a reciprocal functionality to add a ligand;

L is a linking group, which is the same in each of the members of the library;

F is a functional group reactive at an active site of a target enzyme, and is the same reactive functionality in each of the members of the library; and

R is H or a moiety of less than 1kDal providing specific affinity for said target enzymes; the * intends that R is a part of F or L.

5. (Withdrawn) A method according to Claim 4, wherein F is a sulphonyl group and R is other than H and bonded to F.

6. (Withdrawn) A method according to Claim 4, wherein F is a fluorophosphonyl or fluorophosphoryl group.

7. (Withdrawn) A method according to Claim 1, wherein at least one of L and X comprise at least one isotope in unnatural amount, and including the additional step of:

releasing at least a portion of said probe from said conjugate and identifying said portion by means of isotopic difference.

8. (Withdrawn) A method for screening for the bioactivity of a candidate compound toward a group of related target enzymes in a proteomic mixture of proteins from a cell, employing at least one probe, each probe of the formula:

$R^* (F - L) - X$

wherein:

X is a ligand for binding to a reciprocal receptor and/or providing a detectable signal;

L is an aliphatic linking group;

F is a functional group reactive at an active site of a target enzyme; and

R is H or a moiety of less than 1kDal providing specific affinity for said enzymes;

the * intends that R is a part of F or L;

said method comprising:

combining at least one probe with an untreated portion of said mixture and with a portion inactivated with a non-covalent agent under conditions for reaction with said target proteins; sequestering proteins conjugated with said at least one probe from each of said mixtures;

determining the proteins that are sequestered; and

comparing the amount of each of the proteins sequestered from the untreated portion and the inactivated portion as indicative of the bioactivity of said candidate compound with said target proteins.

9. (Withdrawn) A method according to Claim 8, wherein F is a sulphonyl group and R is other than H and bonded to F.

10. (Withdrawn) A method according to Claim 8, wherein F is a fluorophosphonyl or fluorophosphoryl group.

11. (Withdrawn) A method for determining in a proteomic mixture the presence of active target members of a group of related proteins, said related proteins related in having a common functionality for conjugation at an active site, said method comprising:

combining a first portion of said proteomic mixture with at least one activity-based probe comprising a reactive functionality specific for said active site when active, under conditions for conjugation of said probe to said target members;

combining a second portion of said proteomic mixture that has been subjected to non-specific inactivation with said probe(s) under the same conditions used with said first portion of said proteomic mixture;

determining the presence of target members conjugated with said probe(s) in each of said first and second portions of proteomic mixture ; whereby the presence of a greater amount of target members conjugated to said probe(s) in said first portion of said proteomic mixture than in said second, inactivated portion of said proteomic mixture indicates the presence of an active target member.

12. (Withdrawn) A method according to Claim 11, comprising the additional step of characterizing said active target members conjugated with said probe(s).

13. (Withdrawn) A method according to Claim 12, wherein said characterizing comprises degrading said active target member and determining the resulting fractions by mass spectrometry.

14. (Withdrawn) A method according to Claim 11, employing a plurality of activity-based probes having different reactive functionalities specific for different groups of related proteins.

15. (Withdrawn) A method according to Claim 11, wherein said activity-based probes comprise a detectable label.

16. (Withdrawn) A method according to Claim 11, wherein said proteomic mixture is in an intact cell.

17. (Previously presented) A method for determining in a plurality of proteomic mixtures the presence of active target members of a group of related proteins in each of said proteomic mixtures, said related proteins related in having a common functionality for conjugation at an active site, said method comprising:

combining each of said proteomic mixtures in wild-type form with a probe comprising a reactive functionality specific for said active site when active, under conditions for conjugation of said probe to said target members;

determining the presence of target members conjugated with said probe in said proteomic mixtures;

analyzing for the presence of target members conjugated with said probe using simultaneous individual capillary electrokinetic analysis or capillary HPLC;

whereby when said target members are conjugated to target members in said proteomic mixtures, the presence of active target members is determined.

18. (Withdrawn) A method for determining in a plurality of proteomic mixtures the presence of active target members of a group of related proteins, said related proteins related in having a common functionality for conjugation at an active site, said method comprising:

combining a first portion of each of said proteomic mixtures with at least one activity-based probe comprising a reactive functionality specific for said active site when active, under conditions for conjugation of said probe(s) to said target members;

combining a second portion of each of said proteomic mixture that has been subjected to non-specific deactivation, with said probe(s) under the same conditions used with said first portion of said proteomic mixture; and

determining the presence of target members conjugated with said probe(s) in each of said first and second portions of said proteomic mixtures;

whereby the presence of a greater amount of target members conjugated to said probe(s) in said first portion of said proteomic mixtures than in said second, inactivated portion of said proteomic mixtures indicates the presence of active target members.

19. (Withdrawn) A method for determining in a proteomic mixture the presence of active target members of a group of related enzymes, said related enzymes related in having a common functionality for conjugation at an active site, said method comprising:

combining a first portion of said proteomic mixture with at least one activity-based probe comprising a reactive functionality specific for said active site when active, under the same conditions for conjugation of said probe to said target members;

combining a second portion of said proteomic mixture that has been subjected to non-specific inactivation, with said probe(s) under the same conditions used with said first portion of said proteomic mixture;

determining the presence of target members conjugated with said probe(s) in each of said first and second portions of said proteomic mixture;

whereby the presence of a greater amount of target members conjugated to said probe(s) in said first portion of said proteomic mixture than in said second, inactivated portion of said proteomic mixture indicates the presence of an active target member.

20. (Withdrawn) A method according to Claim 19, wherein said probe comprises a ligand and said determining is by binding said ligand in a conjugate to a support and isolating said conjugate.

21. (Withdrawn) A method according to Claim 19, wherein said reactive functionality is a fluorophosphonate or fluorophosphate.

22. (Withdrawn) A method according to Claim 19, wherein said active functionality is an α -haloketone.

23. (Withdrawn) A method according to Claim 19, wherein said active functionality is sulfonate ester or epoxide.

24. (Withdrawn) A method according to Claim 19, wherein said active functionality is a sulfonate ester.

25. (Withdrawn) A method according to Claim 19, wherein said active functionality is α -halohydroxamic acid.

26. (Withdrawn) A method according to Claim 19, wherein said active functionality is an alkyne.

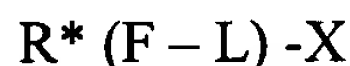
27. (Withdrawn) A method according to Claim 17 or 18, comprising the additional step of characterizing said active target members conjugated with said probe(s) .

28. (Withdrawn) A method according to Claim 27, wherein said characterizing comprises degrading said active target member and determining the resulting fractions by mass spectrometry.

29. (Withdrawn) A method according to Claim 17 or 18, employing a plurality of activity-based probes having different reactive functionalities specific for different groups of related proteins.
30. (Withdrawn) A method according to Claim 17 or 18, wherein said activity-based probe(s) comprises a detectable label.
31. (Withdrawn) A method according to Claim 17 or 18, wherein said proteomic mixture is in an intact cell.
32. (Currently amended) A method according to Claim 17 [or 18], comprising the additional step of characterizing said active target members conjugated with said probe(s).
33. (Previously presented) A method according to Claim 32, wherein said characterizing comprises degrading said active target member and determining the resulting fractions by mass spectrometry.
34. (Currently amended) A method according to Claim 17 [or 18], employing a plurality of activity-based probes having different reactive functionalities specific for different groups of related proteins.
35. (Currently amended) A method according to Claim 17 [or 18], wherein said activity-based probe(s) comprises a detectable label.
36. (Currently amended) A method according to Claim 17 [or 18], wherein said proteomic mixture is in an intact cell.

37. (Currently amended) A method according to Claim 17 [or 18] further comprising the step of analyzing for the presence of proteins conjugated with said probe(s) using simultaneous individual capillary electrokinetic analysis or capillary HPLC.

38. (Currently amended) A method according to Claim [11,] 17[, 18 or 19] wherein said activity-based probe(s) are of the formula:



wherein:

X is a ligand for binding to a ~~reciprocal~~ receptor or a chemically reactive functionality ~~for reacting with a reciprocal functionality to add a ligand;~~

L is a linking group;

F is a functional group reactive at an active site of a target enzyme; and

R is H or a moiety of less than 1kDal providing specific affinity for said target enzymes;

the * intends that R is a part of F or L.

39. (Previously presented) A method according to Claim 38, wherein F is a sulphonyl group and R is other than H and bonded to F.

40. (Previously presented) A method according to Claim 38, wherein F is a fluorophosphonyl or fluorophosphoryl group.

41. (Withdrawn) A method according to Claim 11, wherein at least one of L and X comprise at least one isotope in unnatural amount, and including the additional step of:
releasing at least a portion of said probe from said conjugate and identifying said portion by means of isotopic difference.

42. (Currently amended) A method according to any of Claims [11-13, 15-21,] 17, 32, 33,

35-38, or 40 [or 41] wherein said activity-based probe(s) are fluorophosphonate-biotin (FP-biotin).

43. (Currently amended) A method according to any of Claims [11-13, 15-21,] 17, 32, 33, 35-38, or 40 [or 41] wherein said activity-based probe(s) are FP-peg-biotin.

44. (Currently amended) A method according to any of Claims [11-13, 15-20,] 17, [23, 24,] 32, 33, or 35-39 [or 41] wherein said activity-based probe(s) are selected from the group consisting of 10-((2-pyridylsulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((Benzenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((*p*-Toluenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((4-Methoxybenzenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((Methylsulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((Butylsulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((Octylsulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((4-Nitrobenzenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((8-Quinolinesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((2-Naphthalenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((2-Thiophenesulfonyl)oxo)-N-biotinamidopentyldecanamide, α -undecylenyl alcohol, ((2-pyridylsulfonyl)oxo)-10-undecene, 10-((2-pyridylsulfonyl)oxo)-decanoic acid, 1-(2-pyridylsulfonyl)oxo-octane, 1-(2-pyridylsulfonyl)oxo-ethane, and 1-(methanesulfonyl)oxo-octane.

45. (Previously presented) A method according to claim 44 wherein said activity-based probe is 1-(2-pyridylsulfonyl)oxo-octane.

46. (Currently amended) A method according to Claim [14 or] 34 wherein said activity-based probe(s) are selected from the group consisting of FP-biotin, FP-peg-biotin, 10-((2-pyridylsulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((Benzenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((*p*-Toluenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-((4-Methoxybenzenesulfonyl)oxo)-N-biotinamidopentyldecanamide, 10-

((Methylsulfonyl)oxo)-*N*-biotinamidopentyldecanamide, 10-((Butylsulfonyl)oxo)-*N*-biotinamidopentyldecanamide, 10-((Octylsulfonyl)oxo)-*N*-biotinamidopentyldecanamide, 10-((4-Nitrobenzenesulfonyl)oxo)-*N*-biotinamidopentyldecanamide, 10-((8-Quinolinesulfonyl)oxo)-*N*-biotinamidopentyldecanamide, 10-((2-Naphthalenesulfonyl)oxo)-*N*-biotinamidopentyldecanamide, 10-((2-Thiophenesulfonyl)oxo)-*N*-biotinamidopentyldecanamide, α -undecylenyl alcohol, ((2-pyridylsulfonyl)oxo)-10-undecene, 10-((2-pyridylsulfonyl)oxo)-decanoic acid, 1-(2-pyridylsulfonyl)oxo-octane, 1-(2-pyridylsulfonyl)oxo-ethane, and 1-(methanesulfonyl)oxo-octane.

47. (Withdrawn) A method according to Claim 21 wherein said group of related enzymes is serine hydrolases.

48. (Withdrawn) A method according to Claim 22 wherein said group of related enzymes is cysteine hydrolases.

49. (Withdrawn) A method according to Claim 23 wherein said group of related enzymes is related in having a common functionality comprising at least one of the following: cysteine, histidine, aspartate, and glutamate.

50. (Withdrawn) A method according to Claim 24 wherein said group of related enzymes is alcohol dehydrogenases.

51. (Withdrawn) A method according to Claim 25 wherein said group of related enzymes is metalloenzymes.

52. (Withdrawn) A method according to Claim 26 wherein said group of related enzymes is redox enzymes.